

Hospital wastewater as a hotspot for antibiotic-resistant bacteria and emerging pollutants: A focused systematic review

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Abstract

In the hospital's wastewater, there are groups of un-metabolised and metabolised drugs with disinfectants and diagnostic agents as well as pathogen, ARB, ARGs, and MGEs. Multiple hazards which are occurring will bring about selection, gene transfer and dissemination. Aim of the study: In this paper, we discuss original experimental data published so far (2015 to 15 July 2023) on the joint occurrence, selection, mobility and treatment of ARB/ARGs and emerging chemical pollutants in hospital wastewater. With this, we hope to verify whether the problem of hospital wastewater has reached a stage of crisis like urban wastewater. The study was specifically designed based on PubMed/Europe PMC and publisher/Crossref verification, with Scopus and Web of Science strings supplied for author rerunning before submission. This study was carried out a priori, meaning that it was performed before the actual investigation. Intended to be a focused systematic review, in accordance with PRISMA 2020. According to the study, hospital wastewater was directly sampled in eligible studies and a minimum of two linked domains were reported. For instance, antimicrobial or pharmaceutical residues; phenotypic ARB; ARGs/MGEs; metagenomic hosts; or biological-chemical treatment performance. As many as ten original studies conducted in Asia, Europe and South America met the deliberately stringent evidence-integration criteria. Results were synthesized narratively and by direction of effect rather than pooled statistically as endpoints, denominators and treatment configurations were heterogeneous. Results of the study found in untreated hospital wastewater antibiotic concentrations at ng/L to µg/L scales, diverse multi-drug resistant organisms and clinically important genes. High-priority signals included gene associated with resistance to beta-lactams, sulfonamide, and quinolone, *vanA*, and *int1*. A study proved selecting drug-resistant *E. coli* is possible (9 words) This shows ciprofloxacin and beta-lactams may be tested for selective agents in *E. coli*. Effluent and influent studies showed incomplete removal with conventional treatment. In an opposite manner, the on-site improvement of industrial trains as well as plant-scale ozonation reduced chemical and biological hazards although DNA-based signals and ozone-tolerant survivors continued to be relevant. Based on our analysis, we conclude that hospital wastewater should be regarded as a coupled chemical–biological hotspot, not merely as a pathogen source. Risk management efforts should favour surveillance of the hospital environment, source control in high-risk wards, and multi-barrier treatment with double validation against antibiotic residues, viable ARB, intracellular and extracellular ARGs, MGEs and ecotoxicological endpoints. The protocol was not registered in advance but should be deposited before submission in a version with a date.

Keywords: Antimicrobial Resistance; Antibiotic Resistance Genes; Hospital Effluent; Pharmaceuticals; Contaminants Of Emerging Concern; Resistome; Wastewater Treatment; One Health; PRISMA

1. Introduction

Antimicrobial resistance has a clinical and environmental and structural problems. Hospitals focus the use and excretion of antimicrobials, diagnostic chemicals, disinfectants and the organisms shed by ICU patients. The wastewater therefore

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contains both chemical stressors, and biological carriers within the same matrix. The presence of these compounds in hospital wastewater set them apart from a regular sanitation problem has the property that antimicrobials can impose selection, non-antibiotic chemicals can co-select resistance, dense microbial communities can facilitate horizontal gene transfer, and downstream treatment can reduce absolute loads but still release persistent residues, viable resistant organisms or extracellular DNA [1,2,3].

The term ‘emerging pollutants’ is used here for contaminants which are not presently regulated or monitored consistently although they may have an environmental or health impact. Hospital waste includes antibiotics and other drugs, active metabolites, disinfectants, contrast media and resistance determinants themselves. Antibiotics are especially important because they may still alter competitive fitness and enrich resistant subpopulations below minimum inhibitory concentrations. Rejuvenation cannot be all-encompassing; not one way could lead to the protection of worldwide biodiversity. In this respect, true understanding of chemical measurement and gene detection is paramount. A thorough examination must connect chemical exposure, resistant phenotype, genetic determining and treatment efficacy[4,5,6].

Previous reviews have collected hospital micropollutants or resistance data separately; meta-analyses have summarized the normalized abundance of ARG [7]. The critical unresolved query concerning the extent to which original studies measure the coupled system for the information we obtained has been supported by evidence of experimental selection or evidence of multi- barrier treatment. This review addresses this significant gap with the strict inclusion of ten original studies that integrate at least two evidence domains.

1.1. Review questions and objectives

- The main review question was: In real hospital wastewater systems, are emerging chemical pollutants linked to the occurrence, selection, mobility and environmental release of antibiotic resistant bacteria and their genes? Secondary questions addressed which organisms, genes, and compounds recur; how treatment changes them; and which methodological limitations block quantitative comparison.
- The eligible original studies published between 2015 and 15 July 2026 will be characterized by their geographical and methodological distributions.
- They will also synthesize measured antibiotics/pharmaceuticals, ARB, ARGs and MGEs [mostly correlated with clinically significant resistance].
- Evaluate evidence of either chemical selection or HGT instead of merely assuming causation based on co-occurrence.
- Assessing the efficiency of traditional and advanced treatment based on chemical and biological measures.
- Prepare a research and policy agenda for Q1 that incorporates One Health and source-to-receptor risk pathways.

2. Methods

2.1. Protocol and reporting framework

The manuscript was prepared in accordance with PRISMA 2020. The protocol was not prospectively registered. Before submitting their manuscript, the authors should deposit a dated protocol in an open repository (for example OSF). They must attach the final PRISMA 2020 checklist. Any changes made to the databases, eligibility rules, or the outcome should be declared according to the protocol.

2.2. Eligibility criteria

Table 1 Eligibility Criteria for Hospital Wastewater

Domain	Inclusion criteria	Exclusion criteria
Population/matrix	Untreated or treated wastewater originating from an identifiable hospital/healthcare facility; receiving sewer or water included only when paired with the hospital source.	Municipal, industrial, agricultural or pharmaceutical-manufacturing wastewater without a hospital-specific sample.
Study design	Original field, laboratory, pilot or full-scale experimental research using real hospital wastewater.	Reviews, meta-analyses, protocols, editorials, conference abstracts

		without sufficient data, purely in-silico studies.
Integrated outcomes	At least two linked domains: chemical residues; cultured ARB/phenotypes; ARGs/MGEs; metagenomic hosts; paired biological–chemical treatment performance.	Single-domain studies lacking a link to resistance occurrence, selection, mobility or removal.
Time/language	1 January 2015–15 July 2026; full text in English.	Outside date window or insufficient English information for extraction.
Publication	Peer-reviewed original article with verifiable DOI.	Preprints without peer-reviewed version; unverifiable or duplicated reports.

2.3. Information sources and search strategy

On 15 July 2026 a search through PubMed/Europe PMC and DOI/publisher verification was undertaken. For purposes of reproducibility, the following strings on subscription databases should be rerun by submitting authors and exported in RIS/CSV before submission. There shouldn't be application of citation-date or journal-quartile filter during retrieval

Table 2 Reproducible database search strings

Database	Search string
Scopus	TITLE-ABS-KEY (("hospital wastewater" OR "hospital effluent" OR "healthcare wastewater") AND (antibiotic* OR antimicrobial* OR pharmaceutical* OR "emerging contaminant*" OR micropollutant*) AND (resistan* OR resistome OR "resistance gene*" OR bacteria* OR treatment)) AND PUBYEAR > 2014 AND PUBYEAR < 2027
Web of Science Core Collection	TS= (("hospital wastewater" OR "hospital effluent" OR "healthcare wastewater") AND (antibiotic* OR antimicrobial* OR pharmaceutical* OR "emerging contaminant*" OR micropollutant*) AND (resistan* OR resistome OR "resistance gene*" OR bacteria* OR treatment)); Timespan: 2015–15 July 2026; document type: Article
PubMed/Europe PMC	((("hospital wastewater"[Title/Abstract] OR "hospital effluent"[Title/Abstract]) AND (antibiotic*[Title/Abstract] OR antimicrobial*[Title/Abstract] OR pharmaceutical*[Title/Abstract] OR emerging contaminant*[Title/Abstract])) AND (resistan*[Title/Abstract] OR resistome[Title/Abstract] OR bacteria*[Title/Abstract] OR treatment[Title/Abstract])) AND (2015:2026[pdat])

2.4. Study selection and data extraction

Examination by the integrated-outcome rule experts. We focused on the following fields for each included study for data extraction: (i) country and setting, (ii) wastewater stage, (iii) sampling design, (iv) chemical analytical platform, (v) culture or susceptibility method, (vi) molecular method, (vii) target compounds, organisms, ARGs and MGEs, (viii) treatment configuration, and (ix) principal quantitative or directional findings and limitations. Every final database export and extraction table will be reviewed by a second reviewer before submission. This very evidence set contains ten DOI-verified original studies.

2.5. Risk-of-bias and methodological-quality appraisal

Given that the evidence consisted of environmental cross-sectional surveys pairing treatment studies metagenomic studies and controlled selection experiments no single clinically applicable risk-of-bias tool was relevant. The environmental sample can be appraised by following eight distinctive domains which are (a) clear sampling frame (b) temporal/biological replication (c) validated chemical method (d) appropriate culture/susceptibility method (e) validated molecular workflow (f) negative and positive controls (g) transparent normalization/statistics and (h) conclusions proportionate to the design.

All domains were either suitable, unsuitable, not relevant or vague. Interpretation of this data was moderated by the quality weaknesses of included studies.

2.6. Synthesis strategy

Because of the differences between studies in sampling units, seasons, wastewater stages, analytical detection limits, antibiotic panels, gene targets, normalization denominators and treatment trains, meta-analysis was not attempted. Thematic grouping and direction of effect were used to synthesize results.

The framework applied a causal hierarchy; that is, measured co-occurrence was considered associative; paired influent–effluent changes were interpreted as effects of treatment; and competition or exposure experiments controlling for the latter provide stronger evidence of selection. The concentrations were kept in the units reported by the source studies and were not converted when the underlying denominator or censoring rules were unclear.

3. Results

3.1. Study selection

The ten primary articles of focused evidence were published from 2015 to 2025. The evidence base included Spain, China, Singapore, Netherlands, India, Sweden, Japan and Chile with several multimethod or multicity or multihospital designs. The authors must ensure that the data in the final PRISMA diagram is populated with deduplicated counts from Scopus Web of Science and PubMed exports. The collection of evidence was specifically limited to experiments that linked chemical and biological resistance-related fields or that tested treatment or selection using actual hospital wastewater.

Table 2 PRISMA 2020 flow template for completion after final database export

Stage	Count / disposition
Identification	Records from Scopus: [n]; Web of Science: [n]; PubMed/Europe PMC: [n]; other DOI/publisher verification: [n].
Deduplication	Duplicates removed: [n]; records retained for title/abstract screening: [n].
Screening	Records excluded after title/abstract: [n].
Eligibility	Full texts assessed: [n]; excluded with reasons: single outcome [n], wrong matrix [n], non-original [n], insufficient data [n], duplicate report [n].
Included	Studies included in focused synthesis: 10.

3.2. Characteristics of included studies

Table 3 Data extraction from the ten included original experimental studies

Study; country	Design/matrix	Methods	Key findings	Strength/limitation	DOI
Rodríguez-Mozaz et al., 2015; Spain	Hospital/urban wastewater, WWTP and receiving river	Targeted antibiotics + qPCR ARGs	Fluoroquinolones were highest in hospital effluent; blaTEM, qnrS, ermB, sul1 and tetW peaked in hospital effluent/WWTP influent; antibiotics and ARGs remained detectable after treatment.	Strong source-to-receptor design; heterogeneous removal precluded a single pooled effect.	10.1016/j.watres.2014.11.021
Li et al., 2016; China	Influent/effluent of five hospital WWTPs	Antibiotic quantification + qPCR/integrans	Influent totals: tetracyclines 363.4–753.3 ng/L, sulfonamides 285.5–634.9 ng/L, quinolones 1355.8–1922.4 ng/L. Removal was compound-class dependent; selected ARGs correlated with antibiotics and intI1.	Multihospital comparison; correlations cannot establish direct selection.	10.1007/s11356-016-6688-z
Le et al., 2016; Singapore	Three within-hospital wastewater locations	LC-MS/MS + culture + susceptibility + qPCR	Isolation ward showed the highest ARB and ARG burdens; phenotypic resistance correlated with blaKPC, aac(6')-Ib, sul1/sul2, dfrA, qnrA and intI1.	Integrated chemical/phenotypic/genetic design; single tropical hospital system.	10.1128/AAC.01556-16
Wang et al., 2018; China	Untreated wastewater from three hospitals	SPE-UPLC-MS/MS + culture/high-throughput sequencing + high-capacity qPCR	Six of 14 antibiotics reached µg/L; Escherichia and Acinetobacter dominated cultured multiple-resistant bacteria; high concentrations/diversity of ARGs and MGEs were detected.	Broad integrated profiling; untreated snapshots limit fate inference.	10.1016/j.scitotenv.2017.10.128
Paulus et al., 2019; Netherlands	Two cities; one with advanced on-site HWW treatment	711-compound screening + multiplex qPCR of 13 ARGs, intI1 and 16S	HWW had ~25% more detected antibiotics and higher gene signals than communal wastewater. MBR–ozone–GAC–UV removed 12/19 detected antibiotics versus up to 1/21 under urban treatment; blaKPC	Powerful natural comparison; city-level contextual differences remain possible.	10.1016/j.ijheh.2019.01.004

			and vanA fell below detection on site.		
Marathe et al., 2019; India	Hospital sewage effluent and receiving treatment pathway	Shotgun metagenomics + functional screening	Hospital effluent harbored novel carbapenemases and integron-borne ARGs, demonstrating that clinically relevant and previously undescribed resistance can leave hospitals.	High-resolution discovery; limited temporal replication and abundance-to-risk translation.	10.1186/s40168-019-0710-x
Kraupner et al., 2021; Sweden	Hospital effluent, municipal WWTP influent and effluent	Three controlled E. coli selection assays + chemical analysis	Sterile-filtered HWW strongly selected multidrug-resistant strains among 149 isolates; ciprofloxacin and β -lactams were candidate drivers. Municipal effluent showed no selection in the assays.	Strongest causal selection evidence; one setting and model species.	10.1016/j.envint.2021.106436
Azuma et al., 2022; Japan	Plant-scale batch ozone treatment of HWW	Culture/metagenomics + targeted antimicrobials	Nine antimicrobials (373 ng/L–27 μ g/L) were detected and 96–100% removed after 40 min ozone; most bacteria were inactivated, but resistant survivors and DNA signals require attention.	Full-scale relevance; ozone by-products and regrowth need fuller assessment.	10.3390/antibiotics11070862
Talat et al., 2023; India	Six rural/urban hospitals across northern India	Shotgun metagenomics, read- and assembly-based resistome analysis	Aminoglycoside, macrolide, carbapenem, trimethoprim and sulfonamide ARGs predominated; mcr-5.1 and blaNDM-1 were detected, often on plasmids and in ESKAPE-relevant hosts.	Geographically broad resistome mapping; single time-point samples and no paired chemistry.	10.1128/spectrum.04102-22
Aguilar-Rangel et al., 2025; Chile	Untreated tertiary-hospital outlet	HPLC + culture/CLSI susceptibility + metagenomics	Paracetamol and amoxicillin were quantified alongside diverse culturable bacteria, resistance phenotypes, resistance genes and potential pathogens, establishing a South American integrated baseline.	Triangulated methods; exploratory single-site design and limited chemical panel.	10.3390/antibiotics14111111

3.3. Chemical pollutants and selection pressure

Among the studies, antibiotics were present but often as mixtures from multiple therapeutic classes. Fluoroquinolones were repeatedly prominent hospital-associated residues. Rodríguez-Mozaz et al. found the highest concentrations among all antibiotic families for fluoroquinolones in hospital effluent. In contrast, Li et al. [8]. reported quinolone totals exceeding the corresponding tetracycline and sulfonamide totals in five Chinese hospital systems. Researchers Wang and colleagues measured fourteen antibiotics and found six of them to be present at level $\mu\text{g/L}$ at the hospital wastewater which was untreated. The concentrations of antimicrobials that are environmentally relevant are those below clinical MICs because resistance selection can occur at these concentrations. However, not all environmental communities have minimum selective concentrations that are compound-specific.

The evidence allows for three interpretations. Initially, measured residues show exposure. Secondly, the correlations between compounds and genes imply a common source or selection, but not between. Third, the competition experiments done by Kraupner et al. showed direct preferential survival or enrichment of multidrug resistant *E. coli* in hospital waste. Ciprofloxacin and β -lactams were implicated by the chemical analysis but other mixture effects and non-antibiotic stressors could not be precluded. Experimental correlations allow selection in an experimental system. Thus, causal language may be justified for selection in that experimental system, but not so for every field correlation.

Pharmaceuticals that are not antibiotic-based remain under-researched. Both these studies were the only microbicide specifications among those screened for these combinations after Christensen et al. who did a study on nitazoxanide. This limitation creates a blind spot around various disinfectants, metals, contrast agents, cytostatics, metabolites and transformation products, which may exert toxicity, co-selection or mixture effects.

3.4. Antibiotic-resistant bacteria and clinically relevant hosts

Studies dependent on culture recovered resistant Gram-negative and Gram-positive organisms as well as in sequencing. The presence in the environment of organisms like *Escherichia* and *Klebsiella* contributes to their virulence. Survey revealed *Escherichia* and *Acinetobacter* to be dominant components of the cultured multi-antibiotic resistant fraction. [9] correlated the counts of resistant bacteria with the respective gene abundances within a tropical hospital, with the isolation ward showing the strongest signal. In various hospital samples, Talat et al. allocated clinically essential genes to the strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*.

The studies reveal why culture sequencing should be combined. Culture effectively verifies viability and facilitates resistance testing, however, excludes non-culturable organisms and relies on selective conditions. Shotgun metagenomics captures a broader resistome and can identify genetic context but DNA detection does not alone constitute viability, expression or transmissibility. Therefore, a Q1-level monitoring framework should validate viable counts, phenotypic susceptibility, quantitative genes and genome-resolved host assignment[10].

3.5. Resistance genes, mobile elements and horizontal transfer potential

Recurring genetic pattern seen in common, last-resort antibiotics. They continually identified β -lactamases and determinants of aminoglycoside, macrolide, tetracycline, quinolone (*qnr*), and sulfonamide (*sul1/sul2*). The research resulted into findings from which carbapenemases and mobile colistin resistance were the most clinically significant. In Singapore and the Netherlands, blaKPC appeared associated with hospital discharge; Marathe et al. found novel carbapenemase candidates and integron-borne resistance in Indian hospital effluent while Talat et al. detected blaNDM-1 in four of six samples and reported mcr-5.1 in Indian hospital sewage.

The class 1 integron integrase (*intI1*) which consistently differentiated with ARGs has been observed useful for differentiation of anthropogenic pollution and gene-capture potential. The presence of plasmids, transposable elements, and integron contexts lend credibility to the assumption that resistant genes could be easily mobilized. However, being present in assemblies does not indicate effective transfer. Resolution of mobility and persistence requires conjugation or transformation assays, long-read sequencing and extracellular-extracellular DNA fractionation[11].

3.6. Treatment performance and residual risk

Conventional therapy reduced numerous signals that were chemical and genetic but did not consistently remove them. Rodríguez-Mozaz et al. showed the persistence to the effluent of WWTP and receiving water. [12] showed that the performance was better with tetracyclines than sulfonamides or quinolones. A decline in the

overall number of gene copies may occur alongside a stable or increasing amount if more of the susceptible biomass is removed more efficiently. Studies should therefore report both absolute and normalized measures.

When using advanced source treatment, you see the most cross-domain benefits consistently. In the study conducted in the Netherlands, it was shown that the MBR–ozone–GAC–UV train led to a larger removal of antibiotic resistance genes than conventional urban activated sludge. Moreover, it was able to remove nearly all detected antibiotics and antibiotic resistance genes, and the hospital-associated genes *blaKPC* and *vanA* were reduced to below detection. A Japanese plant-scale ozone study showed 96–100% removal of nine selected antimicrobials after 40 minutes, with good bacterial inactivation. The evidence is there to support multi-barrier treatment, but the absence of risk for a single-process discharge must also be verified: eDNA, transformation products, ozone-tolerant survivors, re-growth and energy and material burdens.

3.7. Methodological quality and certainty

Table 3 Summary methodological appraisal

Evidence domain	General judgment	Main concern for certainty
Sampling frame	Mostly adequate	Several studies used one hospital or few sampling dates; seasonality and flow-proportional sampling were uncommon.
Chemical analysis	Adequate where performed	Target lists and detection limits differed; non-target screening and transformation products were rare.
Phenotypic ARB	Moderate	Panels and breakpoints varied; culture conditions select only part of the community.
ARG/MGE measurement	Moderate-to-high	qPCR panels differ; relative versus absolute normalization limits comparison; metagenomic detection does not prove expression.
Experimental causality	High for one study, otherwise low-to-moderate	Most evidence is cross-sectional; Kraupner et al. uniquely tested selection in controlled assays.
Treatment inference	Moderate	Paired designs were informative, but treatment trains, hydraulic conditions and endpoints were heterogeneous.
Overall certainty	Moderate	Direction of evidence is consistent, but quantitative magnitude and generalizability remain uncertain.

4. Discussion

4.1. Principal interpretation

According to the findings from ten studies, hospital wastewater is a coupled hotspot. The term “hotspot” is not merely justified by a high concentration of resistant bacteria or mobile genetic elements but rather by a confluence of four distinct properties: enrichment in antibiotic and pharmaceutical mixtures, viable multidrug resistant organisms, clinically relevant ARGs found in mobile contexts, and conditions able to select for resistant phenotypes. This convergence was observed across different settings, with different absolute burden and treatment context in different settings.

Every molecule of antibiotic did not directly create every gene we detected is the most defensible causal chain. Hospital activities rather generate a concentrated mix of residuals and microorganisms that can preferentially suppress susceptible bacteria, enrich resistant populations and provide for persistence and gene exchange. The mixture has been modified by treatment through removal, transformation, concentration in sludge or release. Each link needs a unique measurement. Thus, studies that rely on only one endpoint provide an incomplete risk characterization.

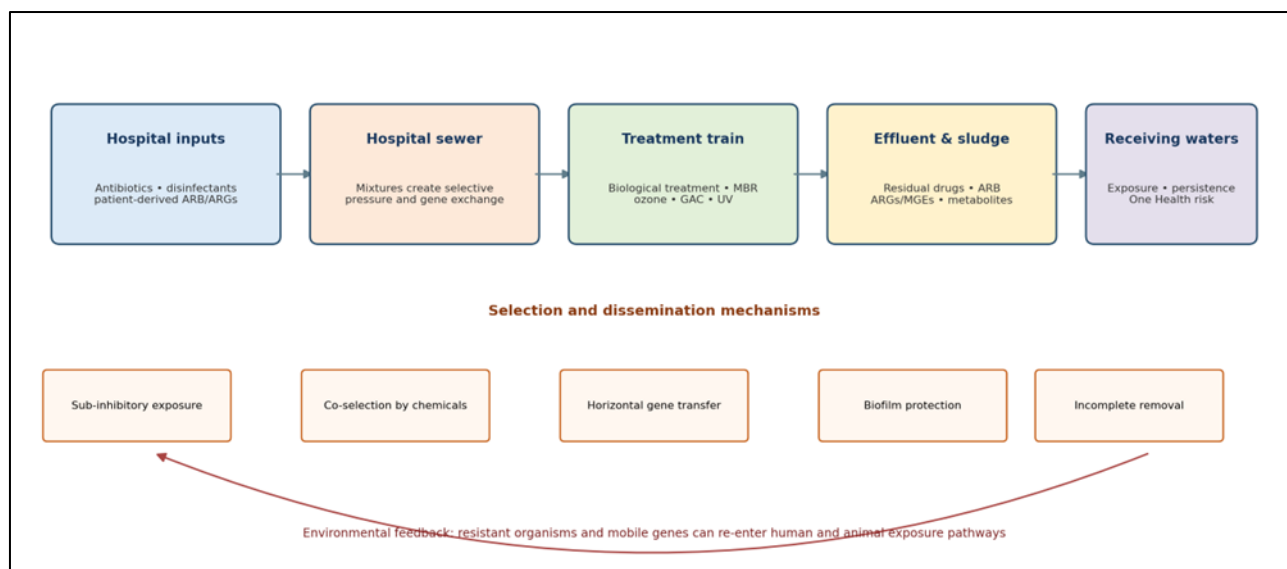


Figure 2 Conceptual source–pathway–receptor model for coupled chemical and antimicrobial-resistance hazards in hospital wastewater.

4.2. Why a meta-analysis was not appropriate

Pooling will create false precision. Studies of chemical composition used different target panels and censoring rules; microbiological studies used different media, antibiotic breakpoints and denominators; qPCR normalised either per volume or to 16S rRNA genes; metagenomic studies used different databases and thresholds; treatment studies differed in hydraulic loading, process sequence and sampling. If future studies are to report on raw concentrations, detection limits, flow, absolute and relative ARG abundance, paired biological replicates, standardized log-removal meta-analysis may become possible.

4.3. Implications for surveillance

A working surveillance panel should be tiered. A minimum level would measure flow, basic water quality, certain antibiotics, and viable *E. coli* and Enterobacterales were tested for resistance against sentinel β -lactamases, 16S rRNA, *sul1*, *int1*. The addition of *blaKPC*, *blaNDM*, OXA-type carbapenemases, *vanA*, *mcr* variants and ward-specific sampling to hospital-risk tier. A higher level will incorporate high dimensional non-target high-resolution mass spectrometry, shotgun/long read metagenomics, eDNA, effect-based selection assays. Outcomes must be presented as loads per day as well as concentrations, which lessens the probability that dilution will be confused with control.

4.4. Implications for treatment and policy

Reviewed evidence supports source-oriented treatment in which hospitals produce unique high-risk discharges. Every hospital need to have the same train. The design must be devised based on risk involving the bed capacity and related factors such as infectious disease bed oncology service use of antibiotics, sewage connection vulnerability of downstream reuse and receiving water. By segregating, we could create wards or streams where the high-risk targets are prioritized. MBR, ozonation, activated carbon and UV could then stress complementary targets. The verification must consist of workable ARB, intracellular and extracellular ARGs, compounds and transformation products not just conventional biochemical oxygen demand or fecal ones[13,14].

Regulatory standards mainly continue to be concentration based and pathogen based. A more effective way to outline regulations generally would be a combination of discharge limits for priority antibiotics effect-based thresholds that relate to resistance selection log-reduction targets viables resistant organisms and monitoring sentinel ARGs/MGEs. It makes sense to control sources and be transparent about surveillance until internationally accepted thresholds of effect are available, particularly where effluent is reused for irrigation or discharged in low-flow waters[15].

4.5. Research agenda

- Take 24-hour flow-proportional composite samples every season and report the daily mass load.
- Quantify antibiotic use, environmental residues and resistance development in the same sampling window.
- Employ absolute qPCR/digital PCR along with 16S-normalized abundance and viability-informed methods.

- Isolate ARG fractions from inside and outside the cell and assess transformation and conjugation potential.
- Linking ARGs, plasmids, and hosts through both short and long read sequences.
- Beyond antibiotics, assess the levels of disinfectants, metals, contrast agents, cytostatics, and metabolites.
- Use paired controls, hydraulic data, energy/cost, by-products, regrowth, sludge pathways to evaluate treatment trains
- Establish a connection between environmental measurements and probabilistic exposure or quantitative microbial risk assessment without assuming gene detection equals infection risk[16,17].
- Widen proof from Middle East, Africa and Latin America using harmonized protocols and open data.

4.6. Strengths and limitations of this review

One of the key strengths of the work is its integrated evidence rule: the papers included connect the chemical, phenotypic, genetic or treatment domain rather than treating any of these in isolation. The synthesis distinguishes association from experimental selection and identifies validated multi-barrier interventions. The proof extends across several continents and analytical platforms.

One important limitation is that it is a narrow ten-study synthesis rather than a broad quantitative review. The independent re-run and documentation of the subscription-database search and exact PRISMA counts prior to submission are essential. Publication bias may arise from English-language and DOI requirements. Diversity stopped combined impact results. Some conclusions are based on single-site studies, and it is possible that there is publication bias towards detection of positive results. Environmental detection does not straight away measure human infection risk[18,19].

5. Conclusion

Hospital effluents continuously present an amalgamation of chemical and biological hazards with them. These include antimicrobial and pharmaceutical residues, viable resistant bacteria, clinically important ARGs and mobile genetic elements. The most compelling evidence shows both the widespread co-occurrence of and directly selective pressure on multidrug-resistant *E. coli* in controlled experiments. Conventional treatment has a variable effect while advanced on-site multi-barrier systems have broader efficacy to remove residues and resistance markers. Hospital wastewater should use a coupled One Health control point for future surveillance and regulation and interventions should be validated for chemical concentration, biological viability, gene mobility and downstream exposure.

Compliance with ethical standards

Acknowledgments

We thank the staff of Ramadi Hospital for their cooperation.

Disclosure of conflict of interest

The author(s) declare(s) that there is no conflict of interest.

Statement of ethical approval

This is not applicable as it synthesises study literature.

Author contributions

Employ CRediT taxonomy and add contributions.

Data availability

All the information generated in the review is available in the cited references and extraction table. Prior to publication, deposit the final database export, screening log and extraction workbook in an open repository

Protocol registration

Not prospectively registered. A dated protocol should be archived before submission.

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